Patent
Attorney Docket: 267/173

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Elaine Weidenhammer et al.

Serial No.: Not Assigned

Filed: Herewith

For: **IMPROVED METHODS FOR GENE EXPRESSION MONITORING ON ELECTRONIC MICROASSAYS**

Group Art Unit: Not Assigned

Examiner: Not Assigned

PRELIMINARY AMENDMENT

BOX Patent Application
Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the subject application, please amend same as follows:

IN THE SPECIFICATION:

Please amend the Title to read as follows:

PRIMER EXTENSION DETECTION METHODS ON
ACTIVE ELECTRONIC MICROARRAYS

Please insert the following paragraph on page 1, after the Title:

-- This application is a divisional of co-pending U.S. Patent Application Serial Number 09/710,200, filed on November 9, 2000, and is also a continuation-in-part of co-pending

CERTIFICATE OF MAILING (37 C.F.R. §1.10)

I hereby certify that I have a reasonable basis to expect that this paper (along with any referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as 'Express Mail - Post Office To Addressee' in an envelope addressed to Commissioner for Patents, Washington, DC 20231.

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Date of Deposit: February 12, 2002

Micheal A. Smith

U.S. Patent Application Serial Number 09/490,965, filed on January 24, 2000, which is a continuation of U.S. Patent Application Serial Number 08/271,882, filed July 7, 1994, now U.S. Patent Number 6,017,696. –

Please replace Table 2 on pages 31-36 with the following Table:

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TABLE 2

Oligonucleotides Used in the Above Experimental Procedures, Organized by Target Gene

Target Gene	**Primer name and description**	**Primer sequence**
Angiotensinogen	bAt7AngBpm.s1—biotinylated chimeric T7 promoter/gene specific oligonucleotide	BiotinTAATACGACTCACTATAGG GAGACACAGAACTGGATGTTGC TGCTGGAG [SEQ. ID NO. 1]
Angiotensinogen	cAng.a1—capture for RNA	BiotinCATGAACCTGTCAATCTTCT [SEQ. ID NO. 2]
Angiotensinogen	pAng.a1—3' gene specific primer	GGAAGGTGCCCATGCCAGAGA [SEQ. ID NO. 3]
Cathepsin G	bAt7CathBpm.s1—biotinylated chimeric T7 promoter/gene specific oligonucleotide	BiotinTAATACGACTCACTATAGG GAGAGCTGCCTTCAAGGGGGAT TCTGGAG [SEQ. ID NO. 4]
Cathepsin G	pCath.a1—3' gene specific primer	AGCTTCTCATTGTTGTCCTTATC C [SEQ. ID NO. 5]
Cathepsin G	cCath.a1—capture for RNA	BiotinTGTTACACAGCAGGGGGC CT [SEQ. ID NO. 6]
c-jun	bT7jun.s1—biotinylated chimeric T7 promoter/gene specific oligonucleotide	BiotinTAATACGACTCACTATAGG GAGACGGCCAACATGCTCAGGG AACAGGT [SEQ. ID NO. 7]
c-jun	cjun.s1—capture for cDNA	BiotinCAAACATTTTGAAGAGAGA CCGTCG [SEQ. ID NO. 8]
c-jun	pjun.a1—3' gene specific primer	TTTTTCTTCGTTGCCCCTCAGCC [SEQ. ID NO. 9]
COX1	pcox1.as.3 —gene specific primer for generation of 500bp fragment	TGCCCAGGATTGATTCACAGG [SEQ. ID NO. 10]

Target Gene	Primer name and description	Primer sequence
COX1	pcox1.as.2—gene specific primer for generation of 250bp fragment	AGGCCAGAAGGAATGATGGG [SEQ. ID NO. 11]
COX1	pcox1.as.1—gene specific primer for generation of 100bp fragment	CTAAGCCCAAAGTGTGGATC [SEQ. ID NO. 12]
COX1	T7.cox1.s—chimeric T7/gene specific oligonucleotide	GAAATTAATACGACTCACTATAG GGAGAACCCTTTTCTCAGGACCT CTGGAGG [SEQ. ID NO. 13]
COX1	cCOX1bpm.a1—capture for RNA	BiotinACAGAGGTCCTGAGAAAAG GGTCT [SEQ. ID NO. 14]
COX2	bAt7COX2bpm.s2—biotinylated chimeric T7 promoter/gene specific oligonucleotide	BiotinTAATACGACTCACTATAGG GAGACTATGAATCATTTGAAGAA CTTACTGGAG [SEQ. ID NO. 15]
COX2	cCOX2.a2—capture for RNA	BiotinCTGCAGACATTTCCTTTTCT [SEQ. ID NO. 16]
COX2	pCOX2.a2—3' gene specific primer	GCATCTGGCCGAGGCTTTTCTAC [SEQ. ID NO. 17]
GAPDH	pGAPs.2—gene specific primer for generation of 500 bp fragment	GTTCGACAGTCAGCCGCATCTTC [SEQ. ID NO. 18]
GAPDH	pGAPs.3—gene specific primer for generation of 100 bp fragment	TGATGCCCCCATGTTCGTCATGG [SEQ. ID NO. 19]
GAPDH	pGAPs.4—gene specific primer for generation of 250 bp fragment	CTTCCAGGAGCGAGATCCCTCC [SEQ. ID NO. 20]
GAPDH	T7GAPbpm.a1—chimeric T7/gene specific oligonucleotide	GTAATACGACTCACTATAGGGCG GGGTGCTAAGCAGTTGGTGGTG CTGGAG [SEQ. ID NO. 21]
GAPDH	cGAPbpm.s1—capture for aRNA	BiotinCAGCCTCAAGATCATCAGC AATGCCT [SEQ. ID NO. 22]
GAPDH	bAt7GAPbpm.s2—biotinylated chimeric T7 promoter/gene specific oligonucleotide	BiotinTAATACGACTCACTATAGG GAGACTCAAGGGCATCCTGGGC TACACTGGAGCAC [SEQ. ID NO. 23]
GAPDH	pGAPDH.a6—3' gene specific primer	GAGGTCCACCACCCTGTTGCTG TAG [SEQ. ID NO. 24]

Target Gene	Primer name and description	Primer sequence
GAPDH	cGAPbpm.a2—capture for RNA	BiotinGTTGAAGTCAGAGGAGACC ACCTGGTGCT [SEQ. ID NO. 25]
HMG-17	bAt7HMG17Bpm.s1—biotinylated chimeric T7 promoter/gene specific oligonucleotide	BiotinTAATACGACTCACTATAGG GAGAGGAATAACCCTGCAGAAA CTGGAG [SEQ. ID NO. 26]
HMG-17	pHMG17.a1—3' gene specific primer	CCCTTCCCCCAAAAACAACAATG A [SEQ. ID NO. 27]
HMG-17	cHMG17.a1—capture for RNA	BiotinCCTGGTCTGTTTTGGCATC T [SEQ. ID NO. 28]
Interleukin 6	pIL6s.4—gene specific primer for generation of 500bp fragment	ATTCTGCCCTCGAGCCCACCGG G [SEQ. ID NO. 29]
Interleukin 6	pIL6S.3—gene specific primer for generation of 250bp fragment	CAAACAAATTCGGTACATCCTCG [SEQ. ID NO. 30]
Interleukin 6	pIL6S.2—gene specific primer for generation of 100bp fragment	TGGATTCAATGAGGAGACTTGCC [SEQ. ID NO. 31]
Interleukin 6	T7IL6bpm.a1—chimeric T7/gene specific oligonucleotide	GTAATACGACTCACTATAGGGCG CCTCACTACTCTCAAATCTGTTC TGGAG [SEQ. ID NO. 32]
Interleukin 6	cIL6bpm.s1—capture for aRNA	BiotinGGAGTTTGAGGTATACCTA GAGTACCT [SEQ. ID NO. 33]
Interleukin 6	bT7IL6.s1—biotinylated chimeric T7 promoter/gene specific oligonucleotide	BiotinTAATACGACTCACTATAGG GAGACCTGAGGGCTCTTCGGCA AATGTAG [SEQ. ID NO. 34]
Interleukin 6	cIL6.s1—capture for cDNA	BiotinAATGGGCATTCCTTCTTCT GGTCAG [SEQ. ID NO. 35]
Interleukin 6	pIl6.a1—3' gene specific primer	GAACAACATAAGTTCTGTGCCCA GTG [SEQ. ID NO. 36]
Interleukin 1 beta	bT7IL1.s1—biotinylated chimeric T7 promoter/gene specific oligonucleotide	BiotinTAATACGACTCACTATAGG GAGACAGAAAACATGCCCGTCTT CCTGG [SEQ. ID NO. 37]
Interleukin 1 beta	cIL1.s1—capture for cDNA	BiotinGCGGCCAGGATATAACTGA CTTCAC [SEQ. ID NO. 38]
Interleukin 1 beta	pIl1.a1—3' gene specific primer	TCCACATTCAGCACAGGACTCTC TG [SEQ. ID NO. 39]

Target Gene	Primer name and description	Primer sequence
LD78	bAt7LD78Bpm.s1—biotinylated chimeric T7 promoter/gene specific oligonucleotide	BiotinTAATACGACTCACTATAGG GAGAAGTGACCTAGAGCTGAGT GCCTGGAG [SEQ. ID NO. 40]
LD78	pLD78.a1—3' gene specific primer	CTCTCAGAGCAAACAATCACAAA CACAC [SEQ. ID NO. 41]
LD78	cLD78.a1—capture for RNA	BiotinTCGAAGCTTCTGGACCCCT [SEQ. ID NO. 42]
Osteopontin	bAt7OstBpm.s1—biotinylated chimeric T7 promoter/gene specific oligonucleotide	BiotinTAATACGACTCACTATAGG GAGAGAGGTGATAGTGTGGTTT ATGGACTGGAG [SEQ. ID NO. 43]
Osteopontin	pOst.a1—3' gene specific primer	CAACGGGGATGGCCTTGTATGC [SEQ. ID NO. 44]
Osteopontin	cOst.a1—capture for RNA	BiotinAACTTCTTAGATTTTGACCT [SEQ. ID NO. 45]
p53	pp53s.3—gene specific primer for generation of 500bp fragment	ACAGAAACACTTTTCGACATAG [SEQ. ID NO. 46]
p53	pp53s.2—gene specific primer for generation of 250bp fragment	AAAGGGGAGCCTCACCACGAGC [SEQ. ID NO. 47]
p53	pp53.s1—gene specific primer for generation of 100bp fragment	CGTGAGCGCTTCGAGATGTTCC [SEQ. ID NO. 48]
p53	T7p53bpm.a1—chimeric T7/gene specific oligonucleotide	GTAATACGACTCACTATAGGGCG ACCCTTTTTGGACTTCAGGTGGC TGGAG [SEQ. ID NO. 49]
p53	cp53bpm.s1—capture for aRNA	BiotinGAGCCAGGGGGGAGCAGG GCTCACT [SEQ. ID NO. 50]
TGFβ1	pTGFb1S.3—gene specific primer for generation of 500bp fragment	GGGATAACACACTGCAAGTGGA C [SEQ. ID NO. 51]
TGFβ1	pTGFb1s.2—gene specific primer for generation of 250bp fragment	CCACGAGCCCAAGGGCTACCAT GC [SEQ. ID NO. 52]
TGFβ1	pTGFb1.s1—gene specific primer for generation of 100bp fragment	CGCTGGAGCCGCTGCCCATCGT GTA [SEQ. ID NO. 53]

Target Gene	Primer name and description	Primer sequence
TGFβ1	T7TGFb1bpm.a1—chimeric T7/gene specific oligonucleotide	GTAATACGACTCACTATAGGGCG GGCGGGACCTCAGCTGCACTTG CTGGAG [SEQ. ID NO. 54]
TGFβ1	cTGFb1bpm.s1—capture for aRNA	BiotinCAGCTGTCCAACATGATCG TGCGCT [SEQ. ID NO. 55]
TGFβ2	bT7TGFb.s1—biotinylated chimeric T7 promoter/gene specific oligonucleotide	BiotinTAATACGACTCACTATAGG GAGACTCTGCCTCCTCCTGCCT GTCTGC [SEQ. ID NO. 56]
TGFβ2	cTGFb.s1—capture for cDNA	BiotinCGGCATCAAGGCACAGGG GACCAGT [SEQ. ID NO. 57]
TGFβ2	pTGFb.a1—3' gene specific primer	CTTCAACAGTGCCCAAGGTGCT CAA [SEQ. ID NO. 58]
TPOX	TPOX9C—biotinylated synthetic target	BiotinTTAGGGAACCCTCACTGAA TGAATGAATGAATGAATGAATGA ATGAATG [SEQ. ID NO. 59]
TPOX	TPOXcapcomp—Cy3 labeled reporter for TPOX9C	CATTCATTCATTCAGTGAGGGTT CC [SEQ. ID NO. 60]
Vimentin	bAt7VimBpm.s2—biotinylated chimeric T7 promoter/gene specific oligonucleotide	BiotinTAATACGACTCACTATAGG GAGACATCGACAAGGTGCGCTT CCTGGAG [SEQ. ID NO. 61]
Vimentin	pVim.a1—3' gene specific primer	CGCGGGCTTTGTCGTTGGTTAG [SEQ. ID NO. 62]
Vimentin	cVim.a2—capture for RNA	BiotinCAGGATCTTATTCTGCTGC T [SEQ. ID NO. 63]
β-Actin	bAt7Actin.s—biotinylated chimeric T7 promoter/gene specific oligonucleotide	BiotinTAATACGACTCACTATAGG GAGACCCCTTTTTGTCCCCCAAC TGGAGA [SEQ. ID NO. 64]
β-Actin	cActin.a—capture for RNA	BiotinCCAAAAGCCTTCATACATC T [SEQ. ID NO. 65]
β-Actin	pbAa.4—3' gene specific primer	AAGGTGTGCACTTTTATTCAACT GGTCTCAAG [SEQ. ID NO. 66]
β-la	pT7AmpBpm.s1—chimeric T7/gene specific oligonucleotide for generation of short RNA	TAATACGACTCACTATAGGCTGG CTGGTTTATTGCTGATAAATCTG GAG [SEQ. ID NO. 67]
β-la	pAmp.a2—3' primer with poly(dT) tract	T_{30}CCAATGCTTAATCAGTGAGGC ACCTATCTC [SEQ. ID NO. 68]

Target Gene	Primer name and description	Primer sequence
β-Ia	cAmp.a1—capture for RNA	biotin-CGAGACCCACGCTCACCGGCT [SEQ. ID NO. 69]
β-Ia	pT7Amp.s1—chimeric T7/gene specific oligonucleotide for generation of full-length gene	TAATACGACTCACTATAGGGCAC CCAGAAACGCTGGTGAAAGTAA AAG [SEQ. ID NO. 70]
β-Thromboglobulin-like protein gene	bAt7Throm.s1—biotinylated chimeric T7 promoter/gene specific oligonucleotide	BiotinTAATACGACTCACTATAGG GAGAGGAAAACTGGGTGCAGAG GGTTCTGGAG [SEQ. ID NO. 71]
β-Thromboglobulin-like protein gene	pbThrom.a1—3' gene specific primer	GGCAACCCTACAACAGACCCAC AC [SEQ. ID NO. 72]
β-Thromboglobulin-like protein gene	cbThrom.a1—capture for RNA	BiotinAGCCCTCTTCAAAAACTTCT [SEQ. ID NO. 73]

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After page 36, and before the claims, please insert the Sequence Listing, attached hereto as Appendix "A".

Please replace the abstract on page 46 with the following abstract paragraph:

-- The present invention presents techniques useful in methods for gene expression monitoring, and other nucleic acid hybridization assays, that utilize microelectronic arrays to drive the transport and hybridization of nucleic acids. Particularly, methods for detecting the level of sample amplicons using electronically assisted primer extension detection, and utilizing individual test site hybridization controls are provided by the present invention. These methods are particularly useful for hybridization assays in which a plurality of nucleic acids are being assayed, as they eliminate the need for the hybridization of multiple reporter probes to captured nucleic acids on the active electronic microarray.

IN THE CLAIMS

Please cancel, without prejudice to their prosecution in another application, claims 1-53 and 59-72.

Please add new claims 73-88:

73. A method of detecting the extent of hybridization of a plurality of nucleic acids in a sample to a plurality of nucleic acid probes, the method comprising:

(a) electronically hybridizing the nucleic acid in the sample to a plurality of nucleic acid probes bound to a support at a two or more predetermined locations, wherein:

i) the nucleic acids in the sample are electronically hybridized to nucleic acid probes at two or more locations on the support, and

ii) the sequence of at least one nucleic acid probe on a first location is different from the sequence of the nucleic acid probes on a second location.

(b) utilizing the hybridized nucleic acids as a templates in a nucleic acid polymerase reaction to extend the bound probes, whereby a labeled nucleotide is incorporated into the extended probes; and

(c) detecting the labeled nucleotide incorporated into the extended bound probes at the predetermined location.

74. The method of claim 73 wherein the labeled nucleotide comprises a labeling moiety selected from the group consisting of fluorescent moieties, colorigenic moieties, chemiluminescent moieties, and affinity moieties.

75. The method of claim 74 wherein the labeled nucleotide comprises a fluorescent moiety.

76. The method of claim 73 wherein the fluorescent moiety is selected from the group consisting of cyanine dye moieties, Bodipy Texas Red moieties, rhodamine moieties, fluorescein moieties, and cumarin moieties.

77. The method of claim 76 wherein the fluorescent moiety is a cyanine dye moiety selected from the group consisting of Cy5 and Cy3.

78. The method of claim 73 wherein the nucleic acid polymerase reaction is a DNA polymerase reaction.

79. The method of claim 73 wherein the nucleic acid polymerase reaction is a reverse-transcriptase reaction.

80. The method of claim 73 wherein the two or more locations each comprise at least one nucleic acid probe with the same sequence.

81. The method of claim 80 wherein the nucleic acid probe with the same sequence is a control sequence probe.

82. The method of claim 73 wherein the nucleic acids in the sample are electronically hybridized to nucleic acid probes at five or more locations on the support, and the sequence of at least one nucleic acid probe at each of the five locations is different from the sequences of the nucleic acid probes at the other locations.

83. The method of claim 73 wherein the nucleic acids in the sample are electronically hybridized to nucleic acid probes at ten or more locations on the support, and the sequence of at least one nucleic acid probe at each of the ten locations is different from the sequences of the nucleic acid probes at the other locations.

84. The method of claim 73 wherein the nucleic acids in the sample are electronically hybridized to nucleic acid probes at twenty or more locations on the support, and the sequence of at least one nucleic acid probe at each of the twenty locations is different from the sequences of the nucleic acid probes at the other locations.

85. The method of claim 73 wherein the nucleic acids in the sample are electronically hybridized to nucleic acid probes at forty or more locations on the support, and the sequence of at least one nucleic acid probe at each of the forty locations is different from the sequences of the nucleic acid probes at the other locations.

86. The method of claim 54 wherein a control sequence probe is also bound to the support at the location.

87. The method of claim 56 wherein the fluorescent moiety is selected from the group consisting of cyanine dye moieties, Bodipy Texas Red moieties, rhodamine moieties, fluorescein moieties, and cumarin moieties.

88. The method of claim 87 wherein the fluorescent moiety is a cyanine dye moiety selected from the group consisting of Cy5 and Cy3.

REMARKS

Applicants have filed this divisional application off of parent application 09/710,200 in order to pursue the subject matter of claims 54-58, which were subject to a restriction requirement in the parent. Applicants have added new claims 73-88 in order to claim further embodiments of this subject matter. Due to the cancellation of claims 1-53 and 59-72, Ling Wang has been removed as an inventor on this divisional application, leaving Elaine Weidenhammer, Xiao Xu, Michael Heller, and Brenda Kahl as inventors.

Applicants submit herewith a paper Sequence Listing and computer readable copy in compliance with 37 CFR 1.821 through 1.825. The Sequence Listing adds no new matter to the application, and the computer readable copy is identical to the paper copy. References to the Sequence ID Numbers in the application have been added to the specification by the above amendment, as has the paper copy of the Sequence Listing.

If the Examiner has any questions regarding this Application or the Preliminary Amendment, he is invited to contact the undersigned at (949) 567-2305.

Respectfully submitted,

LYON & LYON LLP

Dated: February 12, 2002

By: ______________________
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